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Acetylcholinesterase Activity in Grass Shrimp and Aqueous Pesticide Levels from South Florida Drainage Canals

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Abstract. Freshwater drainage canals in South Florida are utilized to manage water in agricultural, urban, and water conservation areas and, as a result, collect urban and agricultural storm runoff that is discharged into the Atlantic Ocean and Gulf of Mexico. Pesticides in this runoff may be toxic to the biota inhabiting these waters. This study evaluated the effects of contaminants in South Florida canals draining into Biscayne Bay on the estuarine grass shrimp (*Palaemonetes intermedius*), a representative invertebrate species. Results of surface water analysis for pesticides indicated that eight pesticides out of 52 analyzed were detected. The herbicide metolachlor was found at all nine sites in the five canals sampled at concentrations up to 119 ng/L. Atrazine was detected at seven sites at concentrations up to 29 ng/L. Three organophosphate insecticides (chlorpyrifos, malathion, diazinon) were detected at three sites in two canals (Military and North). Grass shrimp from these three sites showed significantly reduced levels of the acetylcholinesterase enzyme as compared to control shrimp. These two canals are similar in the land use areas drained—urban and suburban and agriculture. The results suggest that monitoring organisms for AChE levels can be a means of detecting exposure to organophosphorus pesticide contamination.

In South Florida (US), the Biscayne Bay estuary is the most densely populated estuary of the South Atlantic coast with population distributed along a narrow coastal strip (NOAA 1990). Previous research in Biscayne Bay has shown that areas of relatively high chemical contamination are present in sediments resulting in significant toxicity in laboratory tests (Long *et al.* 1999). Two important factors that may affect the ecosystem of Biscayne Bay and surrounding areas are (1) increased urbanization resulting in increased stormwater runoff and pollutants and (2) the alteration and control of freshwater flow entering the Bay (USGS 2001). Freshwater drainage canals are utilized to manage water in agricultural, urban, and water conservation areas of South Florida. As a result, these canals collect urban and agricultural storm water runoff that is discharged into the Atlantic Ocean and Gulf of Mexico (Miles and Pfeuffer 1997). Pesticides in this runoff may pose a toxicological risk to the biota inhabiting the receiving waters.

Organophosphate and carbamate insecticides produce toxicity in vertebrates and invertebrates by inhibiting the nervous system enzyme acetylcholinesterase (AChE). The inhibition of this enzyme can be used as a biomarker of exposure to these classes of pesticides. Most studies describing the use of AChE levels in aquatic organisms as a biomarker of contamination have been with fish (Bocquene *et al.* 1993; Payne *et al.* 1996; Stien *et al.* 1998; Kirby *et al.* 2000) with fewer reports on aquatic invertebrates (Escartin and Porte 1997; Den Besten *et al.* 2001).

The objective of this study was to evaluate the effects of contaminants in South Florida drainage canals on an estuarine representative invertebrate species. This objective was accomplished by analyzing surface water collected from canals draining into Biscayne Bay and Barnes Sound for pesticides and by analyzing AChE levels in grass shrimp (*Palaemonetes intermedius*) populations from these sites.

Materials and Methods

Grass Shrimp Collection

The study site extended from Princeton Canal in Biscayne Bay, Florida, south to a marina adjacent to US Highway 1 in Barnes Sound

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(Figure 1). Six sites from canals discharging into Biscayne National Park, two sites from a canal discharging into Barnes Sound, and one site within Barnes Sound were sampled for grass shrimp. Site designations, number of shrimp collected and physicochemical water parameters are provided in Table 1. Shrimp were collected live with a dip net from each site in water no more than one meter deep. Shrimp were taken from the net, placed in plastic bags and then frozen immediately in dry ice at the site of collection. The sites with the most shrimp collected indicated sites where the most suitable habitat was present (i.e. abundant submerged vegetation and algae-covered rocks along the shore). The samples were transported to the National Ocean Service laboratory at Charleston, South Carolina, sorted on ice, and identified to species (*Palaemonetes intermedius*). Shrimp were identified according to Williams (1984), as based on Holthuis (1952) and Fleming (1969). The shrimp were then divided into two animals per sample, wrapped in aluminum foil, and placed in a −70°C freezer until assayed for AChE activity. Laboratory reared *P. intermedius* were used as a reference population. This population of *P. intermedius* was an F1 generation obtained from shrimp originally collected in Manatee Bay, Florida. Previous work on acetylcholinesterase (AChE) in *Palaemonetes* had concentrated on the species *P. pugio* (Key and Fulton 1993; Key *et al.* 1998a). As no previous AChE work on *P. intermedius* has been published, a laboratory-reared population of *P. pugio* was also sampled for AChE analysis for comparative purposes. This population of *P. pugio* was an F1 generation obtained from shrimp originally collected in a pristine area of Leadenwah Creek, South Carolina.

Acetylcholinesterase Assay

Whole body AChE activity was measured in grass shrimp using a modification of the method described by Key *et al.* (1998b) as based on the original Ellman procedure (Ellman *et al.* 1961). Each sample analyzed consisted of two adult shrimp. Sample sizes from each site ranged 6–10. Each sample was homogenized (Pro Scientific model Pro 200 motor with a 20- × 150-mm stainless steel generator) on ice in 50 mM Tris-HCl buffer (pH = 8.1) at 20 mg/mL for 45 s. Next, 75 μL of each homogenate was added to a test tube-containing 1.425 mL of Tris-HCl buffer. After a 15 min incubation period at 30°C, 967 μL of the dilute homogenate was added to a cuvette containing 33 μL of 0.87% 5,5′-dithiobis-(2-nitrobenzoic acid), the color reagent. Finally, 10 μL of 75 mM acetylthiocholine, the substrate, was added to the cuvette then covered with parafilm, inverted to mix, and placed in a spectrophotometer to read continuously for 1 min at a wavelength of 412 nm. For each homogenate sample, three subsamples were assayed. A fourth subsample was incubated with 15 μL of 10 μM eserine to account for nonenzymatic hydrolysis of the substrate. The protein content of the homogenate was determined using the Sigma Assay Procedure, a modification of the original Lowry method (Sigma Chemical Co. 1989; Lowry *et al.* 1951). Whole body AChE activity was reported as nmol product formed/mg Protein/min.

Statistical Analysis

The results of the AChE activities were analyzed for normal distribution (Kolmogorov-Smirnov Test) and homogeneity of variance (Bartlett's Test). Statistical analysis of the results was evaluated using ANOVA and Dunnett's Multiple Comparison Test (Gad and Weil 1988). Alpha for all tests was set to 0.05. All statistical analyses used the laboratory reared *P. intermedius* as the control group.

Water Collection and Analysis

Nine sites from canals discharging into Biscayne National Park and two sites from a canal discharging into Barnes Sound were sampled for pesticides (Figure 1). Collection of water and shrimp occurred in December as cultivation of crops and associated pesticide usage occurs in South Florida during the winter months (Miles and Pfeuffer 1997). Water samples were collected concurrently with the grass shrimp samples. Due to lack of suitable *P. intermedius* habitat shrimp were not collected at Sites 1, 3, and 5. A water sample was not collected at Site 12 as the equipment was not available at the time of shrimp collection (Figure 1). Water samples were collected from a depth of 1 m using a submersible marine pump. The pump was joined to a length of Teflon tubing connected to two in-line, stainless steel filter holders housing a 1 μm pore size, multi-grade GMF glass fiber filter (Whatman #1841070) and a 0.7 μm pore size. GF/F filter (Whatman #1825150), respectively. The filtered water passed into a labeled and precleaned, 18-L, stainless steel canister, sealed with an airtight lid. A field blank was collected each sampling day by pumping 10 L of organic free water through the sampling and filtration system into a precleaned stainless steel canister.

The water samples were shipped in coolers with dry ice at the end of each sampling day via overnight mail to the USDA-ARS laboratory in Beltsville, MD. Water samples were refrigerated upon arrival at the laboratory and filtered and extracted within three days of collection. Two 8-L portions of each sample were measured into clean stainless steel canisters for duplicate processing. Each sample canister was pressurized with high purity nitrogen, forcing the water sample through a certified solid phase extraction (SPE) cartridge containing 500 mg of hyper-cross-linked styrene-divinylbenzene copolymer, ENV+ (Jones Chromatography) extraction resin. This type of SPE cartridge has been used in previous studies of pesticides in estuarine surface waters (Lehotay *et al.* 1999). After extraction, the ENV+ cartridge was dried with nitrogen and eluted with certified high purity solvents (6 mL of dichloromethane (DCM) followed by 9 mL of 3:1 acetone:acetonitrile). This 15 mL extract was concentrated to a final volume of 0.5 mL under nitrogen and analyzed. Analyses were carried out using a Varian 3800 GC coupled to a Saturn 2000 ion trap MS operating in splitless mode with a J&W Scientific DB-17MS (50% methyl, 50% phenyl polysiloxane), 30-m, 0.25-mm internal diameter, 0.25-μm film thickness, capillary column. The carrier gas was ultra high purity helium at a constant flow rate of 1.0 mL/min, controlled by a constant flow pressure program. The GC was operated at an injector port temperature of 260°C and an initial oven temperature of 130°C. The temperature program for the GC oven was as follows: 130°C for 1 min, 5°C/min to 280°C, then hold for 6 min at 280°C. The GC-MS interface temperature was maintained at 280°C and the ion trap temperature was 220°C. The ion trap MS operated in selective ion storage mode, scanning for ions with masses of 70–450.

A field blank, matrix spike and matrix spike duplicates were analyzed with each batch of water samples. No interfering peaks were observed in field and laboratory blank samples. The pesticides detected in the water samples along with the instrumental detection limits and percent recovery are given in Table 2.

Results

Average AChE levels in the *P. intermedius* laboratory control population (56.50 nmol/mg P/min) were not significantly different from AChE levels in the *P. pugio* laboratory control population (59.21 nmol/mg P/min; Figure 2). The AChE levels of these laboratory reared populations were also not significantly different from AChE levels in field collected *P. pugio*

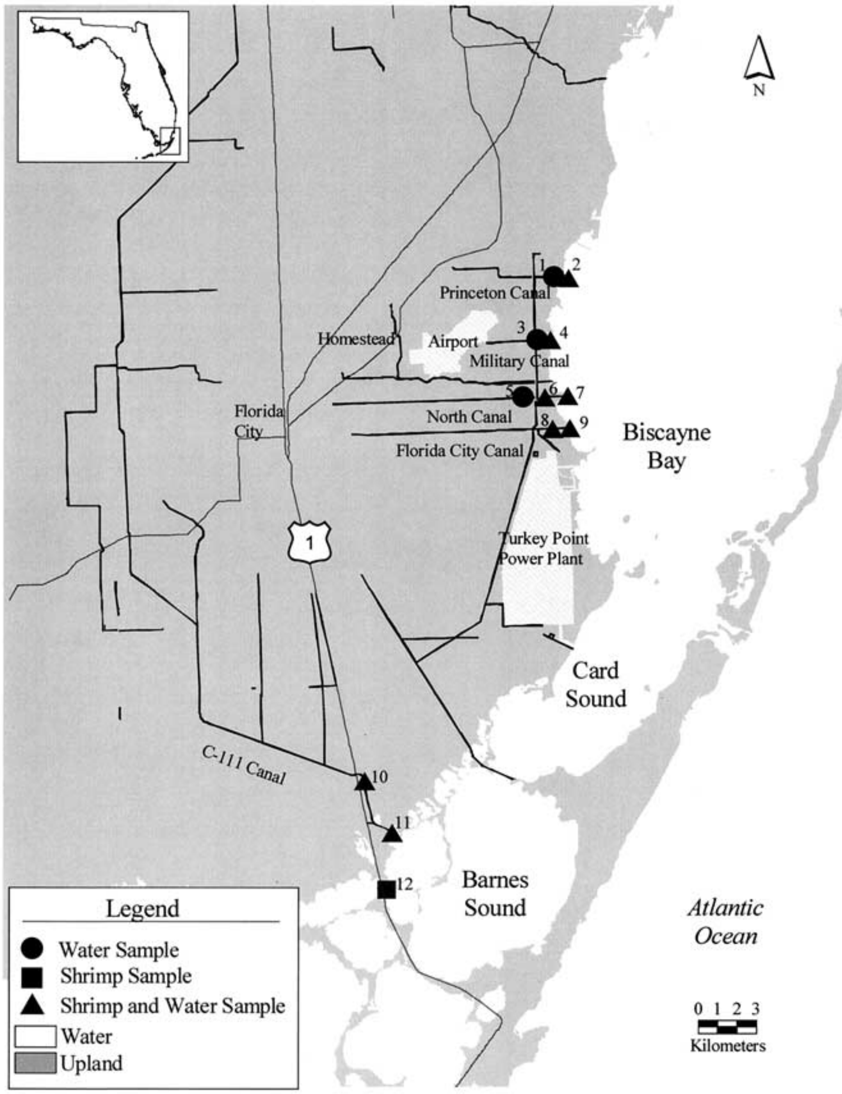

Fig. 1. South Florida drainage canal sample sites for water and shrimp collection

from a pristine area of Leadenwah Creek, SC (56.98 nmol/mg P/min; not shown).

The results of the grass shrimp AChE assays showed three sites with significantly reduced levels of AChE in comparison to the *P. intermedius* laboratory control population (Figure 2). Grass shrimp from Site 6 (North Canal upstream), Site 7 (North Canal mouth), and Site 4 (Military Canal mouth) had AChE levels of 40.89, 44.99, and 47.90 nmol/mg P/min, respectively, compared to the laboratory control AChE level of 56.50 nmol/mg P/min.

Surface water contaminant analysis detected eight pesticides out of 52 analyzed. These contaminants included two herbicides (atrazine and metolachlor), two atrazine metabolites (CEAT and CIAT), three organophosphate insecticides (chlorpyrifos, diazinon, and malathion), and an organochlorine metabolite (p,p′-DDE) (Table 3). The herbicides were the most prevalent compounds with metolachlor present at all sites sampled ranging from 119.1 ng/L at Site 6 (North Canal) to 2.5 ng/L at Site 11 (C-111 Canal). Organophosphates (OP) were detected at three sites, including Site 3 (Military Canal) and Sites 5 and 6 (North Canal). The highest concentration of OPs was measured for diazinon with concentrations up to 60.3 ng/L.

Acute and chronic water quality criteria (WQC) from the US and Canada were available for most of the pesticides detected (Table 3). Atrazine, metalochlor, and p,p′-DDE levels detected, at the time of sampling, were well below WQC values. The chlorpyrifos levels detected in North Canal approached that of the US Environmental Protection Agency's chronic WQC (US EPA 1999). The diazinon level from the same site in North Canal was 75% of the chronic WQC value as determined by the New York State Department of Environmental Conservation (Macdonald *et al.* 1999).

Discussion

These results have shown that AChE levels in *P. intermedius* were depressed at three South Florida drainage canal sites and that pesticide contaminants were present at the five canals sampled. Of the five canals from which water was

Table 1. Number of *P. intermedius* collected, along with water quality measured, at nine South Florida stations

Site	Date	Shrimp Collected	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Salinity (ppt)
Princeton Canal						
Site 2	12/1/99	15	21.4	7.7	4.8	18.9
Military Canal						
Site 4	12/1/99	29	21.8	7.8	5.7	14.3
North Canal						
Site 6	12/6/99	30	24.5	ND	6.7	17.0
Site 7	12/1/99	26	21.3	8.1	9.3	18.3
Florida City Canal						
Site 8	12/2/99	12	21.3	8.3	5.3	18.8
Site 9	12/1/99	26	20.5	8.0	5.3	18.6
C-111 Canal						
Site 10	12/2/99	12	21.2	8.3	4.4	15.6
Site 11	12/2/99	30	19.6	8.4	8.1	16.7
Manatee Bay Marina						
Site 12	12/6/99	24	20.6	ND	6.5	15.1

ND = not determined.

Table 2. Instrumental detection limits and spiked recoveries for pesticides in water samples

Compound	ng/L[a]	% Recovery[b]	Compound	ng/L	% Recovery
acetochlor	6.3	99 ± 23	ethoprop	13	90 ± 21
alachlor	3.1	98 ± 23	fenamiphos	9.5	96 ± 19
aldrin	3.1	71 ± 10	α-HCH[e]	16	86 ± 19
ametryn	6.3	98 ± 24	β-HCH	3.1	98 ± 24
atrazine	3.1	101 ± 22	δ-HCH	3.1	91 ± 21
azinphos-methyl	25	127 ± 146	heptachlor	6.3	83 ± 15
CEAT[c]	16	115 ± 32	heptachlor epoxide	6.3	87 ± 21
α-chlordane	6.3	75 ± 19	malathion	3.1	101 ± 29
γ-chlordane	6.3	76 ± 20	metalaxyl	3.1	98 ± 24
chlorothalonil	3.1	95 ± 21	methoxychlor	16	57 ± 39
chlorpyrifos ethyl	6.3	91 ± 24	metolachlor	3.1	95 ± 24
chlorpyrifos methyl	3.1	99 ± 23	metribuzin	3.1	96 ± 24
CIAT[d]	16	104 ± 24	naled	25	85 ± 39
cyanazine	6.3	93 ± 23	norflurazon	19	128 ± 51
diazinon	3.1	92 ± 21	pendimethalin	3.1	89 ± 27
p,p′-dicofol	9.9	74 ± 21	phorate	13	70 ± 8
dieldrin	3.1	89 ± 25	p,p′-DDD	3.1	73 ± 24
endosulfan sulfate	19	83 ± 31	p,p′-DDE	3.1	53 ± 15
endosulfan I	9.4	85 ± 20	p,p′-DDT	6.3	61 ± 21
endosulfan II	19	81 ± 31	simazine	9.4	94 ± 24
ethion	6.3	79 ± 34	trans-nonachlor	3.1	70 ± 17

[a] Instrumental detection limits.
[b] Mean % recovery ± standard deviation of pesticides spiked into water samples ($n = 4$). Results were adjusted for native pesticide levels.
[c] CEAT = 6-amino-2-chloro-4-ethylamino-*s*-triazine.
[d] CIAT = 6-amino-2-chloro-4-isopropylamino-*s*-triazine.
[e] HCH = hexachlorocyclohexane.

analyzed, two canals (Military and North) had detectable levels of OPs. It was from these two canals that AChE levels in the shrimp assayed were significantly depressed. Sites 3 and 5, which contained the highest levels of OPs, were not ecologically suitable habitats for *P. intermedius* as the sites were freshwater. In addition to the OPs detected, Military and North canals had the highest levels of herbicides and the organochlorine metabolite detected. These pesticides are not, by themselves, known to affect AChE activity in grass shrimp. However, the three OPs detected are well-known grass shrimp AChE inhibitors (Key and Fulton 1993; Key *et al.* 1998b).

In the United States, the most frequently detected herbicides in 40–70% of streams in urban and agricultural areas were atrazine and metolachlor, respectively. The most frequently detected insecticides, nationally, in streams in urban and agricultural areas were diazinon (up to 70% of those sampled), chlorpyrifos (30%), and malathion (20%; Gilliom *et al.* 1999).

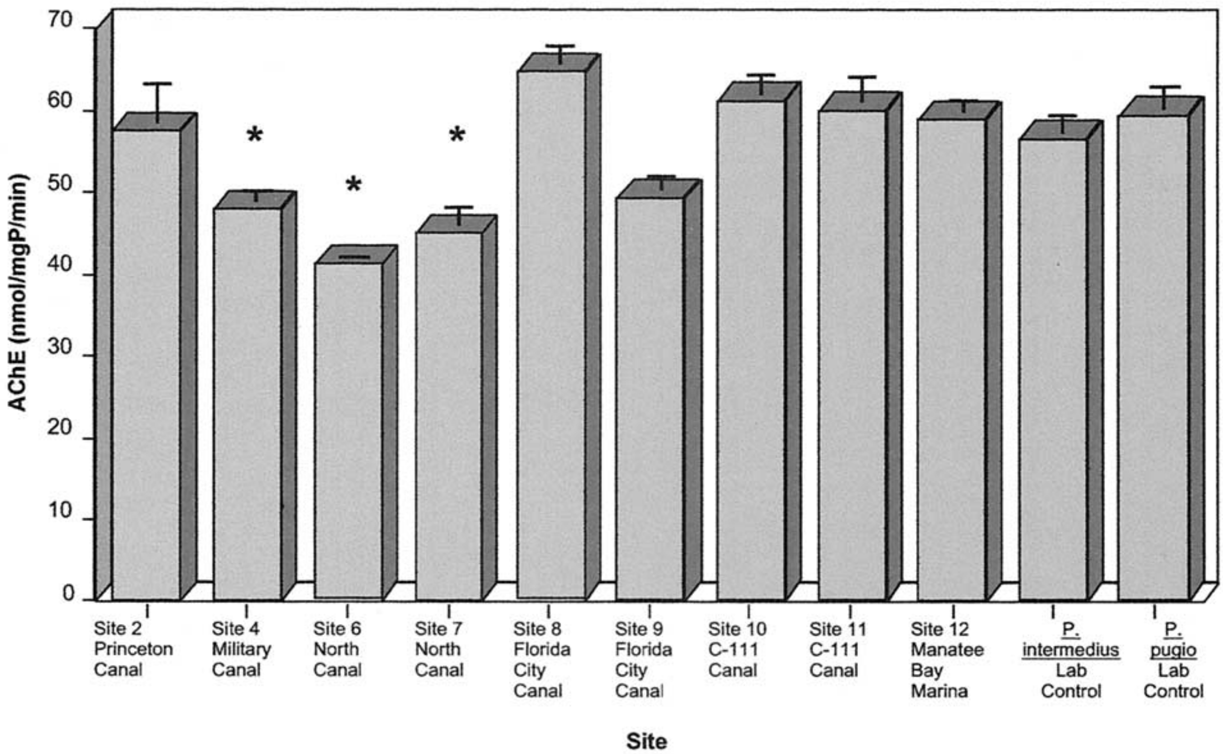

Fig. 2. Average acetylcholinesterase levels in *Palaemonetes intermedius* collected from South Florida drainage canals compared to laboratory control *P. intermedius*. Error bars denote standard error of the mean. *Significantly different from *P. intermedius* laboratory control

As seen in Table 3, these pesticides were also the most frequently detected in this present research.

In the South Florida area studied, the South Florida Water Management District (SFWMD) is the state agency responsible for water resource management. The OP insecticides and herbicides detected in this present study also corresponded to the most frequently applied pesticides in the SFWMD. Chlorpyrifos was used mainly for golf courses and urban residential situations. Diazinon use was for agriculture while malathion use was mainly for mosquito control. Atrazine use was mainly for agriculture while there was no use data for metolachlor (Miles and Pfeuffer 1997). Military and North canals, in which the OPs were detected and AChE levels were reduced, both drain large areas of similar land use—urban and suburban areas of the towns of Homestead and Florida City; and agriculture, including both food and ornamental crops (Figure 1).

Pfeuffer and Matson (2000) analyzed water samples from 36 sites from SFWMD canals. Of the 11 pesticides they detected, nine were herbicides, including five detected in this present study—atrazine, CEAT, CIAT, metolachlor, and diazinon. Atrazine was the most prevalent pesticide, detected in 26 of the 36 sites sampled as compared to metolachlor, this present study's most commonly detected pesticide. Two OPs (diazinon and ethion) were detected by Pfeuffer and Matson (2000) at just three sites. The diazinon level of 60 ng/L detected at those three sites was similar to diazinon levels detected in this present study at Site 5 (Table 3).

Monitoring organisms for AChE levels can be a means of detecting exposure to OP contamination. This may be a better measure of OP contamination due to the relatively short persistence of these compounds in the environment (Habig and Di Giulio 1991). For this present study, at the time of sampling, the levels of the OP compounds detected in the canals were well below acute exposure levels that reduce AChE activity (Cunha Bastos *et al.* 1991; Key and Fulton 1993; Key *et al.* 1998b). The inhibition observed here could be a reflection of earlier grass shrimp exposures to higher OP concentrations. Inhibition could also be due to other compounds present that have been shown to affect AChE levels but were not analyzed in this study such as carbamate insecticides and metals. However, carbamate exposure is generally considered to be reversible and AChE inhibition is likely to undergo spontaneous reactivation (Habig and Di Giulio 1991). AChE inhibition from metal exposures is at concentrations usually found at point sources (Guilhermino *et al.* 1998). Organophosphates are generally irreversible inhibitors because the dephosphorylation rate of the bound enzyme proceeds at an insignificant rate. Therefore, the inhibitory effects of OP exposure may be long lasting with recovery depending on new enzyme synthesis (Habig and Di Giulio 1991). Several studies with shrimp, crab, and lobster species have shown that AChE inhibition in the animals still occurred days after exposure had ended (Habig *et al.* 1986; Reddy and Rao 1988; McHenery *et al.* 1991; Abdullah *et al.* 1994). A slow time course for recovery of depressed AChE levels may cause exposed organisms to be susceptible to other anthropogenic or natural hazards or to exhibit behavior not conducive to maintaining the population.

Another concern with the presence of OPs is their interactive effect with other pesticides. Recently, there have been published reports on the synergistic toxicity found between atrazine and two

Table 3. Concentrations (ng/L) of surface water pesticides in South Florida canals at the time of sampling with corresponding water quality criteria values (ng/L)

SITE	Atrazine	CEAT	CIAT	Metolachlor	Chlorpyrifos	Diazinon	Malathion	p,p′-DDE
Princeton Canal								
Site 1	15.4	12.7*	28.8	8.6	—	—	—	—
Site 2	—	—	—	2.9*	—	—	—	—
Military Canal								
Site 3	17.9	—	35.3	14.9	—	39.2	8.1	—
Site 4	12.3	—	24.4	10.5	—	—	—	—
North Canal								
Site 5	29.4	—	27.9	65.1	5.2*	60.3	—	3.7
Site 6	22.8	—	35.4	119.1	—	10.8	—	—
Site 7	15.2	—	12.2*	8.0	—	—	—	—
Florida City Canal								
Site 8	16.0	—	11.1*	8.9	—	—	—	—
Site 9	7.9	—	14.3*	9.2	—	—	—	—
C-111 Canal								
Site 10	26.3	—	—	12.8	—	—	—	—
Site 11	—	—	—	2.5*	—	—	—	—
Field Blank	—	—	—	—	—	—	—	—
Lab Blank	—	—	—	—	—	—	—	—
Water Quality Criteria								
chronic	26,710[1]	+	+	+	5.6[1]	+	100[1]	+
acute	759,500[1]	+	+	7800[2]	11.0[1]	80[2]	+	14,000[3]

— = Pesticide not detected.
* Pesticide present at levels below quantification limit but full spectral match was made.
[1] Saltwater quality criteria value to which an aquatic community can be exposed without resulting in an unacceptable effect (chlorpyrifos, diazinon: US EPA 1999; atrazine-proposed: Federal Register 2001).
[2] Maximum freshwater concentration that should not be exceeded at any time (Macdonald *et al.* 1999).
[3] Maximum saltwater concentration that should not be exceeded at any time (Macdonald *et al.* 1999).
+Value not determined.

OPs—chlorpyrifos and diazinon. The toxicity of chlorpyrifos to midge larvae (*Chironomus tentans*) was increased four times in the presence of atrazine and the toxicity of diazinon increased two times (Belden and Lydy 2000). Synergism of atrazine with chlorpyrifos was also associated with increased inhibition of AChE in midge larvae (Jin-Clark *et al.* 2002). A comparable trend for decreased AChE activity in midge larvae was found with mixtures of atrazine and malathion but the trend was not statistically significant (Belden and Lydy 2001).

As urban and suburban areas continue to encroach in South Florida and much needed agriculture continues in this fertile region, then pesticide contamination will persist with potential impacts to estuarine fauna. To have a successful pesticide biomonitoring program in such a region, measurements of AChE levels should also be included in resident organisms in waters where acute or chronic OP contamination occurs. Persistent low levels of pesticides, along with the potential for interaction between pesticides of different classes, become a concern with the appearance of sublethal effects in grass shrimp. A better understanding of these effects on aquatic life is needed in order to have a more complete assessment of Biscayne Bay estuarine health.

References

Abdullah A, Kumar A, Chapman J (1994) Inhibition of acetylcholinesterase in the Australian freshwater shrimp (*Paratya australiensis*) by profenofos. Environ Tox Chem 13:1861–1866

Belden J, Lydy M (2000) Impact of atrazine on organophosphate insecticide toxicity. Environ Toxicol Chem 19:2266–2274

Belden J, Lydy M (2001) Effects of atrazine on acetylcholinesterase activity in midges (*Chironomus tentans*) exposed to organophosphorus insecticides. Chemosphere 44:1685–1689

Bocquene G, Galgani F, Burgeot T, Le Dean L, Truquet P (1993) Acetylcholinesterase levels in marine organisms along French coasts. Mar Poll Bull 26:101–106

Cunha Bastos VLF, Cunha Bastos J, Lima JS, Castro Faria MV (1991) Brain acetylcholinesterase as an *in vitro* detector of organophosphorus and carbamate insecticides in water. Wat Res 25:835–840

Den Besten PJ, Valk S, Weerlee E, Nolting R, Postma J, Everaarts J (2001) Bioaccumulation and biomarkers in the sea star *Asterias rubens* (Echinodermata: Asteroidea): A North Sea field study. Mar Environ Res 51:365–387

Ellman G, Courtney K, Andres J, Featherstone R (1961) A new and colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Escartin E, Porte C (1997) The use of cholinesterase and carboxylesterase activities from *Mytilus galloprovincialis* in pollution monitoring. Environ Toxicol Chem 16:2090–2095

Federal Register (2001) Notice of availability of draft aquatic life criteria document for Atrazine. Federal Register 66(187):1–67

Fleming LE (1969) Use of the male genitalic details as taxonomic characters in some species of Palaemonetes (Decapoda, Palaemonidae). Proc Biol Soc Wash 82:443–452

Gad S, Weil C (1988) Statistics and experimental design for toxicologists. Tellford Press, Caldwell, New Jersey

Gilliom R, Barbash J, Koplin D, Larson S (1999) Testing water quality for pesticide pollution. Environ Sci Tech 33:164–169

Guilhermino L, Barros P, Silva M, Soares A (1998) Should the use of inhibition of cholinesterases as a specific biomarker for organophosphate and carbamate pesticides be questioned? Biomarkers 3:157–163

Habig C, DiGiulio R, Nomeir A, Abou-donia M (1986) Comparative toxicity, cholinergic effects, and tissue levels of S,S,S,-tri-n-butyl phosphorotrithioate (DEF) to channel catfish (*Ictalurus punctatus*) and blue crabs (*Callinectes sapidus*). Aquat Toxicol 9:193–206

Habig C, DiGiulio R (1991) Biochemical characteristics of cholinesterases in aquatic organisms. In: Mineau P (ed) Cholinesterase-inhibiting insecticides: Their impact on wildlife and the environment. Elsevier Science Publishers, New York, pp 19–33

Holthuis LB (1952) A general revision of the Palaemonidae (Crustacea Decapoda Natantia) of the Americas. II. The subfamily Palaemoninae. Allan Hancock Foundation Publications 12:1–396

Jin-Clark Y, Lydy M, Zhu K (2002) Effects of atrazine and cyanazine on chlorpyrifos toxicity in *Chironomus tentans* (Diptera: Chironomidae). Environ Tox Chem 21:598–603

Key PB, Fulton MH (1993) Lethal and sublethal effects of chlorpyrifos exposure on adult and larval stages of the grass shrimp, *Palaemonetes pugio*. J Environ Sci Health B28:621–640

Key PB, Fulton MH, Layman SL, Scott GI (1998a) Azinphosmethyl exposure to grass shrimp (*Palaemonetes pugio*) life stages with emphasis on larval acetylcholinesterase activity. Bull Environ Contam Toxicol 60:645–650

Key PB, Fulton MH, Scott GI, Layman SL, Wirth EF (1998b) Lethal and sublethal effects of malathion on three life stages of the grass shrimp, *Palaemonetes pugio*. Aquat Toxicol 40:311–322

Kirby M, Morris S, Hurst M, Kirby S, Neall P, Tylor T, Fagg A (2000) The use of cholinesterase activity in flounder (*Platichthys flesus*) muscle tissue as a biomarker of neurotoxic contamination in UK estuaries Mar Poll Bull 40:780–791

Lehotay SJ, Harman JA, McConnell LL (1999) Agricultural pesticide residues in oysters and water from two Chesapeake Bay tributaries. Mar Poll Bull 37:32–44

Long ER, Sloane G, Scott G, Thompson B, Carr S, Biedenbach J, et al. (1999) Magnitude and extent of chemical contamination and toxicity in sediments of Biscayne Bay and vicinity. NOAA Technical Memorandum NOS NCCOS CCMA 141

Lowry O, Rosebrough N, Farr A, Randall R (1951) Protein measurements with the folin phenol reagent. J Biol Chem 193:265–275

Macdonald D, Berger T, Wood K, Brown J, Johnsen T, Haines M, et al. (1999) A compendium of environmental quality benchmarks. Environment Canada, Georgis Basin Ecosystem Initiative, GBEI 99-01

McHenery JG, Saward D, Seaton D (1991) Lethal and sub-lethal effects of the salmon delousing agent dichlorvos on the larvae of the lobster (*Homarus gammarus* L.) and herring (*Clupea harengus* L.). Aquaculture 98:331–347

Miles CJ, Pfeuffer RJ (1997) Pesticides in canals of South Florida. Arch Environ Contam Tox 32:337–345

National Oceanic and Atmospheric Administration (1990) Estuaries of the United States. Vital statistics of a national resource base. Strategic Assessment Branch, Ocean Assessments Division, Rockville, Maryland

Payne JF, Mathieu A, Melvin W, Fancey L (1996) Acetylcholinesterase, an old biomarker with a new future? Field trials in association with two urban rivers and a paper mill in Newfoundland. Mar Poll Bull 32:225–231

Pfeuffer RJ, Matson F (2000) Pesticide surface water quality report. August 2000 sampling event. South Florida Water Management District, West Palm Beach, Florida

Reddy MS, Rao KR (1988) *In vivo* recovery of acetylcholinesterase activity from phosphamidon and methylparathion induced inhibition in the nervous tissue of penaeid prawn (*Metapenaeus monoceros*). Bull Environ Contam Toxicol 40:752–758

Sigma Chemical Company (1989) Sigma diagnostics protein assay (Procedure No. P5656). Sigma Chemical Co., St. Louis, Missouri

Stien X, Percic P, Gnassia-Barelli M, Romeo M, Lafaurie M (1998) Evaluation of biomarkers in caged fishes and mussels to assess the quality of waters in a bay of the NW Mediterranean Sea. Environ Poll 99:339–345

U S Environmental Protection Agency (1999) National recommended water quality criteria—correction. EPA 822-Z-99-001. U S Environmental Protection Agency, Office of Water, Washington, DC

US Geological Survey (USGS) (2001) Ecosystem history of Biscayne Bay and the Southeast coast. http://sofia.usgs.gov/publications/fs/145-96 Accessed 20 March 2001

Williams AB (1984) Shrimps, lobsters, and crabs of the Atlantic coast of the eastern United States, Maine to Florida. Smithsonian Institution Press, Washington, DC